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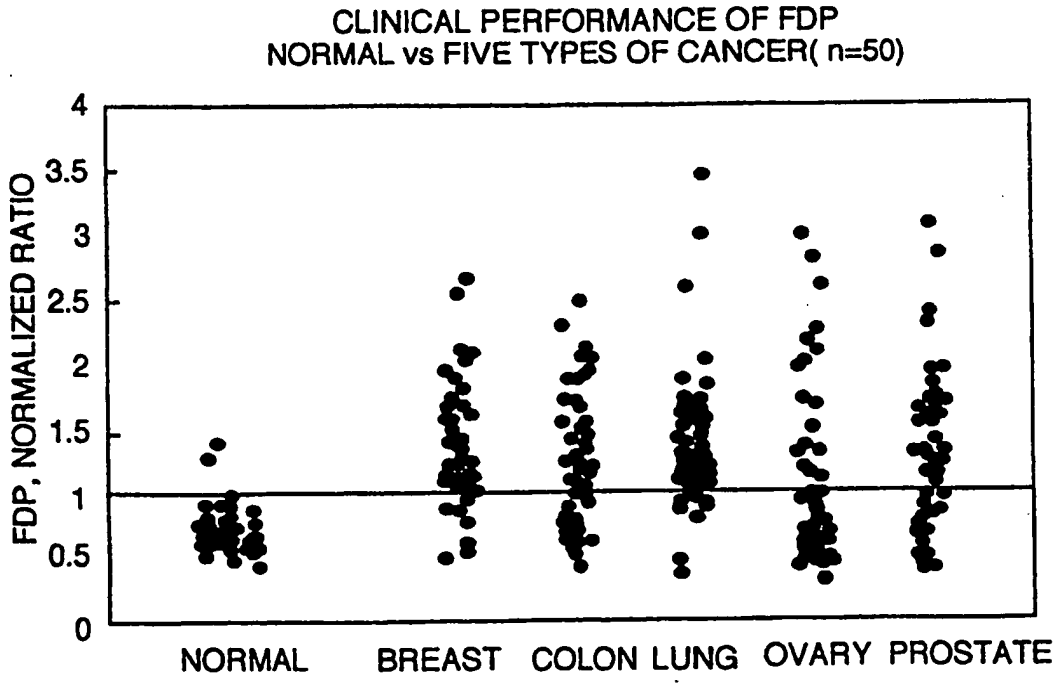

(57) Abstract

The present invention relates to a broad cancer immunoassay. Specifically, an immunoassay for peptides associated with oncogenic processes such as metastatic proteolysis is disclosed. In an illustrative embodiment, the immunoassay utilizes antibodies which bind to peptides which are generated by protelytic processes and which contain epitopes which are masked in undegraded blood proteins such as fibrinogen. Detection of such degradation peptides in a biological sample by immunol gical methods allows the diagnosis f a wide variety of cancers.

IMMUNOASSAY FOR THE DETECTION OF CANCER

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/048,405, entitled "IMMUNOASSAY FOR THE DETECTION OF CANCER," filed on June 3, 1997, by Ngo et al., and U.S. Provisional Application No. 60/060,088 entitled "IMMUNOASSAY FOR THE DETECTION OF CANCER," filed on September 26, 1997, by Ngo et al., which are incorporated by reference herein.

Background of the Invention

1. Field of the Invention.

This invention relates generally to immunoassays for the detection of cancer.

2. Description of Related Art.

An ongoing challenge in medicine is the development of methods that permit the rapid and accurate diagnosis of disease. Despite recent advances in diagnostic technologies, current techniques for the diagnosis of many diseases are either inadequate or cost prohibitive for a wide scale application. One such illustrative disease is cancer. Many "cancer antigens" have been discovered, for example: cancer antigens CEA, CA19-9 and CA242 are used in the diagnosis and treatment of gastrointestinal cancer; cancer antigen CA125 is used in the diagnosis and treatment of ovarian cancer; cancer antigen AFP is associated with testicular and liver cancers; the CA15-3 and HER2/neu antigens are associated with breast cancers; and the PSA and PAP antigens have been shown to be associated with prostate cancer. While the identification of such antigens can be useful once a patient is identified for being at risk for specific cancer or has been diagnosed with a specific cancer, they are of limited use in identifying individuals with cancer in a general population. A general screening of the population using specific cancer antigens would be expensive due to the multiple tests required and would only detect the specific cancers for which antigens are available.

Some antigens, such as the carcinoembryonic antigen, are found in patients with a number of different cancers, such as lung, liver, pancreas, breast, head or neck, bladder, cervix and prostate, in addition to those suffering from adenocarcinoma of

associated with the proteolytic activity associated with malignancy should yield new markers that are associated with oncogenic processes.

There is a need in the art for the identification of antigens which are associated with universal oncogenic processes, and which are not limited to a specific type of cancer. Such pan-marker or universal marker antigen(s) will be useful for the routine screening of patients to determine if they have cancer. After an initial screening, patients with elevated concentrations of the pan-marker, when compared to a "normal" population, would be further screened to determine if they do in fact have cancer and the specific type of cancer from which they are suffering. Additionally, it is desirable that such a pan-marker is present in blood, or other biological fluids, so that testing can be performed on easily obtainable samples.

Summary of the Invention

The present invention is directed to immunoassays for the detection of cancers. In one embodiment, the invention provides a method for detecting cancer in a subject by contacting a biological sample obtained from the subject with an antibody that binds an epitope on a blood protein degradation peptide that is masked in the blood protein and determining the presence of an antibody-peptide complex. In a preferred embodiment of the invention, the blood protein is human fibrinogen and the antibody recognizes an epitope comprising the amino acids 15 to 21 of the β-chain of human fibrinogen.

A wide variety of assays for the degradation peptide may be utilized. In one embodiment, the assay is an enzyme-linked immunoadsorbent (ELISA) assay. In a preferred embodiment, the assay is a sandwich type ELISA immunoassay. Biological samples which are assayed in the present invention may be obtained from a variety of sources. In a preferred embodiment, the biological sample consists of human blood. In addition, a variety of modifications and variations of this assay are disclosed. In one embodiment, the assay includes the additional step of screening a biological sample isolated from the subject for the presence of a second tumor marker. In a preferred embodiment of this variation, the second tumor marker consists of either PSA, CEA, CA 15-3, CA 19-9 or CA 125, or a combination thereof.

A significant feature of the invention is the identification of cancer markers which comprise epitopes on endogenous proteins that are usually inaccessible to immunodetection in normal subjects. In a number of the exemplary embodiments, the

FIG. 10c. is derived from a non-reduced gel and the probe was derived from a ring shaped particle extract.

FIG. 10d. is derived from a non-reduced gel and probed with a monoclonal antibody of the invention.

FIGs. 11a-c show the selectivity of the assay through graphs of the standard curves for fibrinogen fragment D, fibrinogen and fibrinogen fragment E as a function of absorbance at 450 nm.

Detailed Description of the Invention

Definitions

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies and variations thereof including antibody fragments, chimeric or other recombinant molecules that are known in the art. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

The term "tumor marker" as used herein is broadly defined as any one of a wide variety of peptides, nucleic acids and related molecules of which the presence or levels of are used to assess the status of oncogenic processes.

The term "masked" as used herein, for example in the context of degradation peptides, is broadly defined as peptide sequences that are not appreciably recognized or accessible by detection systems, such as antibodies, in normal endogenous proteins. A "masked" peptide may exist within the interior of a native protein, but is not exposed until the protein is degraded and an internal peptide is released or exposed.

The term "degradation peptide" as used herein is broadly defined as a peptide fragment of a larger protein which has been degraded, for example, as occurs with the proteolytic degradation of blood proteins that is observed in oncogenic processes.

metalloproteinase inhibitors prevents metastasis of lung cancer cells. One consequence of the release of proteases by tumor cells into the bloodstream is the proteolysis of serum proteins such as fibrinogen. Therefore, the extent of proteolytic degradation of serum proteins can be correlated with the activity of the tumor cells. Quantitatively the degree of proteolysis can be determined by measuring the quantity of the degradation products generated by the action of the proteases. This measurement is, therefore, an indirect estimate of the degree of malignancy of the tumor cells.

A significant feature of the invention is the identification of cancer markers which comprise epitopes on endogenous proteins that are generally inaccessible to immunodetection. Specifically, while these epitopes are usually masked by the factors such as the 3 dimensional structure of the protein, they become unmasked and accessible to immunodetection for example, upon proteolytic degradation that occurs in oncogenesis. With this knowledge, methods which measure unique epitopes that are either sterically or immunochemically unreactive in the native fibrinogen molecule and are manifested secondary to proteolytic degradation of fibrinogen are of particular interest. Further, in view of the concurrent increase in the formation of fibrin and in the secretion of proteases in malignant conditions, the measurement of serum fibrinogen degradation product (FDP) levels may represent a useful measure of malignancy. Specifically, methods to detect proteolytic degradation products of fibrinogen and other plasma proteins with minimal interference from the parent protein (the protease substrate) are of particular interest for use in a cancer detection assay. The results of studies establishing the viability of an immunoassay, called Oncochek, for the detection of FDPs as indicators of the presence of various cancers is described herein.

Within the present invention, peptides associated with oncogenic processes may be found in detectable concentrations in the biological samples of warm-blooded animals, including humans, possessing a disease which disrupts epithelial tissue. As disclosed in the present invention, unmasked peptides may be indicative of a variety of diseases and are detectable in a variety of samples, with or without purification of such peptides. For example, degradation peptides are shown to be associated with invasive cancers. Invasive cancers include cervical, urogenital (e.g., bladder and prostate), lung, colorectal, and head and neck cancers. Such peptides are also

a peptide is isolated from a sample and contacted with an appropriate detection antibody. Complexes may be isolated by capture on a solid support (e.g., heparin agarose or polystyrene or heparin coated on polystyrene) or with a "capture" antibody prior to or simultaneous with a "detection" antibody. In another embodiment, peptide–antibody immunocomplexes are formed between an antibody and a peptide, without prior purification of the complex. Incubation of a sample with an antibody is under conditions and for a time sufficient to allow immunocomplexes to form. Detection of complexes or polypeptide constituents by immunological means is also amenable to quantification where it is desired to determine the amount of a peptide.

Detection of one or more immunocomplexes formed between a peptide and an antibody specific for the peptide may be accomplished by a variety of known techniques, including radioimmunoassays (RIA) and enzyme linked immunosorbent assays (ELISA). The immunoassays known in the art include the double monoclonal antibody sandwich immunoassay technique of David et al. (U.S. Pat. No. *4,376,110*); monoclonal–polyclonal antibody sandwich assays (Wide et al., in Kirkham and Hunter (eds.), Radioimmunoassay Methods, E. and S. Livingstone, Edinburgh, 1970); the "western blot" method of Gordon et al. (U.S. Pat. No. *4,452,901*); immunoprecipitation of labeled ligand (Brown et al., J. Biol. Chem. 255: 4980–4983, 1980); enzyme–linked immunosorbant assays as described by, for example, Raines and Ross (J. Biol. Chem. 257: 5154–5160, 1982); immunocytochemical techniques, including the use of fluorochromes (Brooks et al., Clin. Exp. Immunol. 39: 477, 1980); and neutralization of activity (Bowen–Pope et al., Proc. Natl. Acad. Sci. U.S.A. 81: 2396–2400, 1984), all of which are hereby incorporated by reference. In addition to the immunoassays described above, a number of other immunoassays are available, including those described in U.S. Pat. Nos.: *3,817,827*; *3,850,752*; *3,901,654*; *3,935,074*; *3,984,533*; *3,996,345*; *4,034,074*; 4,098,876, and 5,591,595, all of which are herein incorporated by reference.

For detection purposes, the antibodies may either be labeled or unlabeled. When unlabeled, the antibodies find use in agglutination assays. In addition, unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the antibody, such as antibodies specific for immunoglobulin. Alternatively, the antibodies can be directly labeled. Where they are labeled, the reporter group can include radioisotopes, fluorophores, enzymes, luminescers, or dye particles. These and other labels are well known in the art and are

molecule, and then (c) detecting the presence or absence of the reporter group. An example of a molecule capable of binding to the immunocomplex is protein A.

It will be evident to one skilled in the art that a variety of methods for detecting the immunocomplex may be employed within the present invention. Reporter groups suitable for use in any of the methods include radioisotopes, fluorophores, enzymes, luminescers, and dye particles.

Taking advantage of the foregoing information, a method for detecting proteolytic degradation products of plasma proteins with minimal interference from the parent protein (the protease substrate) has been devised and used as a cancer detection assay. Specifically the method measures unique epitopes that are manifested secondary to proteolytic degradation of fibrinogen. These epitopes are either sterically or immunochemically unreactive in the native fibrinogen molecule. In addition to providing a general cancer assay, the invention provides a method for monitoring the course of a neoplastic condition by quantitatively determining the presence of peptides present in a biological sample over time.

The present invention is directed at a method for measuring the quantity of proteolytic degradation products of serum proteins. To overcome interference from undegraded, native serum proteins, a peptide contained within the interior of the native proteins is used. Such peptides are "masked" in the native protein and are not recognized or accessible by detection systems, such as antibodies, when the protein is intact. These "masked" peptides are not exposed until the protein is degraded and the internal peptides are released or exposed.

In one embodiment of the present invention, the method measures proteolytic degradation of fibrinogen with minimal interference from intact fibrinogen. In this embodiment of the present invention, two different antibodies are used as the detection system. One of the antibodies is specific for the peptide GHRPLDK which is part of the amino acid sequence of the β–chain of fibrinogen, located near its amino terminus.

Assay specificity is achieved by the use of two different antibodies in a two–site, solid–phase enzymometric assay. The more highly specific antibody, which is immobilized to the solid phase consists of a murine monoclonal to a glycine–histidine–arginine–proline–leucine–aspartate–lysine–cysteine (GHRPLDKC) octapeptide. The first seven amino acids of this peptide represent an internal sequence within the β–chain of fibrinogen, which is near the amino terminus and is exposed

As illustrated in the Examples below, assays for the peptides described above can be combined with tests for the presence of one or more known organ-specific tumor markers to increase the clinical sensitivity and enhance the diagnostic capacity of these assays. Such combination assays may be performed at the same time or sequentially. Those skilled in the art appreciate that there are a wide variety of known organ-specific tumor markers which are associated in varying degrees with different cancer lineages and which may be utilized in conjunction with the assays described herein (see e.g. Lamerz et al., "Serum Marker Combinations in Human Breast Cancer", In Vivo 7(6B): 607-613 (1993). When used in conjunction with the recognized organ-specific tumor marker for breast, colon, and lung cancers the unique epitope detected by the Oncochek immunoassay system appears to offer increased clinical sensitivity.

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The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized. All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated.

Example 1

Coating of Antibody onto 96-Well Microtiter Plates

The monoclonal anti-fibrinogen-peptide antibody (Clone D1G1OVL2) was dissolved and diluted to 2 µg/ml in pH 8.8 borate buffer (0.125 M Borate, pH 8.8, 0.225 M NaCl, 5 mM EDTA, 50 mM 3-amino-m-caproic acid, 10 µg/ml 4-aminobenzamidine-HCl). 120-µl aliquots of the diluted antibody solution were added to each well of each microtiter plates (96-well microtiter plates obtained from Fisher Scientific, Fair Lawn, NJ) and incubated overnight (15-20 hr) at 25°C.

The microtiter plates where then washed twice with Tris buffered saline, pH 7.4 (TBS: 2.5 mM Tris, pH 7.4, 13.7 mM NaCl, 0.3 mM KCl, 0.002% (v/v)

Gaithersburg, MD) was then added to each well, and the plates were incubated for 15 minutes at 25°C. At the end of the incubation 100 µl stop solution (0.1 M HCl) was added to each well. The solution in the wells of the microtiter plates was then read at 450 nm.

Example 4

Statistical Analysis

The assay sensitivity–specificity relationship was analyzed using ROC (receiver–operating characteristic) plots that were constructed by measuring the levels of FDP from sera of both cancer patients and normal control subjects. Such an analysis is a powerful means to describe diagnostic accuracy of the assay. The diagnostic sensitivity is defined by equation 1:

Sensitivity = True Positives/(True Positives + False Negatives)

and the diagnostic specificity is defined by equation 2:

Specificity = True Negatives/(True Negative + False Positives)

The comparative ability of fragment D, fragment E, intact fibrinogen and FDP to form sandwiches between the monoclonal and polyclonal antibodies are summarized in Table I.

Example 5

Calibration Curve

The calibrators for the assay were prepared by plasminolysis of fibrinogen as described in Example 2. Intact fibrinogen (fibrinogen not subjected to prior treatment with plasmin) was unreactive in the assay of the present invention whereas immunoreactive FDP were formed from fibrinogen by plasmin treatment in a time–dependent fashion (Table I).

Example 6

Studies Using Clinical Samples

Sera from fifty control patients (non-cancer) and sixty-five cancer patients were obtained from Orange Coast Hematology and Oncology Groups, Poland Institute of Oncology, Austin Medical Ventures, and LA Metropolitan Hospital. The segmentation of the cancer patient group included 12 lung cancer patients, 10 breast cancer patients, 11 prostate cancer patients, 18 ovarian cancer patients, and 14 colon cancer patients.

Table II shows the FDP levels in the sera of the 50 normal (Table IIa) control subjects and the 65 cancer patients (Table IIb).

Table II(a)

Measurement Of The FDP Levels In

Control Subjects (Non-Cancer)

Sample #	Gender	D_m/F^1 Assay (μg/ml FDP)	Sample #	Gender	D_m/F^1 Assay (μg/ml FDP)
1	M	33	26	F	215
2	F	19	27	F	50
3	F	86	28	F	207
4	F	27	29	F	0
5	F	91	30	F	133
6	F	35	31	F	59
7	F	14	32	U[2]	57
8	F	12	33	F	96
9	F	26	34	M	0
10	F	69	35	M	122
11	F	0	36	M	102
12	F	30	37	F	160
13	F	3	38	M	103
14	F	0	39	M	0
15	F	0	40	M	99
16	F	96	41	M	71
17	F	0	42	M	0

Sample #	Gender	D_m/F Assay (μg/ml FDP)	Sample #	Gender	D_m/F^1 Assay (μg/ml FDP)
Lung Cancer Patents			Ovarian Cancer Patients		
Breast Cancer Patients			13	F	125
1	F	14	14	F	167
2	F	0	15	F	195
3	F	0	16	F	162
4	F	81	17	F	154
5	F	215	18	F	144
6	F	0	Colon Cancer Patients		
7	F	101	1	F	0
8	F	0	2	M	0
9	F	0	3	M	510
10	F	0	4	F	9
Prostate Cancer Patients			5	M	134
1	M	113	6	M	0
2	M	192	7	F	211
3	M	0	8	F	222
4	M	345	9	M	80
5	M	17	10	M	236
6	M	251	11	M	17
7	M	371	12	M	0
8	M	129	13	F	47
9	M	167	14	M	52
10	M	270			
11	M	451			

Samples from cancer patients generally exhibited higher concentrations of FDP, using the D_m/F assay format, than did control patients.

Based on the data presented in Table II, an ROC analysis of the assay was performed to obtain information on the relationship between the sensitivity and specificity of the assay. The result of the ROC analysis is presented in Table III which

The results shown in Tables II and III demonstrated that the assay of the present invention is capable of detecting more than one type of cancer with a high degree of specificity and an acceptable degree of sensitivity.

Example 7

Clinical Performance of FDP Relative to Other Markers

Sera from control patients (non-cancer) and from patients with breast, colon, lung, ovarian or prostate cancer were obtained from a commercial supplier. Fifty samples were used in each group.

FDP levels were measured and normalized such that a normalized ratio of 1.0 represents the upper limit of the normal range. Figure 3 shows the results of these measurements for each group.

Levels of known cancer antigens were also measured in the same samples and these levels were compared to the normalized ratios of FDP. Figure 4 is a scatterplot of CA 15-3 levels as compared to FDP normalized ratio for individual samples from breast cancer patients. Figure 5 is a scatterplot of CA19-9 levels as compared to FDP normalized ratio for 22 of the 50 individual samples from colon cancer patients. Figure 6 is a scatterplot of CEA levels as compared to FDP normalized ratio for 28 of the 50 individual samples from colon cancer patients. Figure 7 is a scatterplot of CEA levels as compared to FDP normalized ratio for individual samples from lung cancer patients. Figure 8 is a scatterplot of CA 125 levels as compared to FDP normalized ratio for individual samples from ovarian cancer patients. Figure 9 is a scatterplot of PSA levels as compared to FDP normalized ratio for individual samples from prostate cancer patients.

These scatterplots demonstrate the increased sensitivity of FDP measurements relative to measurement of other cancer antigens. This increased sensitivity is particularly demonstrated by the datapoints which fall within the lower right quadrant of the plots. The results presented in Figure 3 show that FDP measurements detect a wide variety of cancers.

Results of the Oncochek assay indicate that FDP levels in the sera of patients with various types of cancer are significantly elevated in comparison to normals. For example, FDP levels in the sera of normal control subjects were compared with those in the sera of patients with five types of cancers. Each group consisted of 50 patients and included breast, colon, lung, ovarian, and prostate cancers. The data presented in

Figures 10a–d show results from reduced (with mercaptoethanol; Figs. 10a–b) and non–reduced (without mercaptoethanol; Figs. 10c–d) gels. In Figures 10a and 10c, the probe was derived from a ring shaped particle extract. (Ring shaped particles are described in United States Patents Nos. 5,635,605, issued June 3, 1997, and 5,459,035, issued October 17, 1995.) In Figures 10b and 10d, the probe was the monoclonal antibodies of the invention.

These results show that the molecules of the invention can be used to detect cancer with a much higher specificity than obtained with other cancer detection probes.

The above description is of one embodiment of the present invention. However, it will be clear to those skilled in the art that various changes and modifications may be made without departing from the spirit of the invention.

12. The method of claim 11, wherein the antibody recognizes an epitope comprising the amino acids 15 to 21 of the β–chain of human fibrinogen.

13. The method of claim 12, wherein the antibody is a monoclonal antibody.

14. The method of claim 11, wherein the presence of the antibody–peptide complex is determined by an assay comprising an enzyme–linked immunoadsorbent assay.

15. The method of claim 14, wherein the antibody is immobilized to a solid support.

16. The method of claim 15, wherein the enzyme–linked immunoadsorbent assay comprises a capture immunoassay wherein the antibody–peptide complex is detected with a second antibody which binds the peptide.

17. The method of claim 16, wherein the second antibody is joined to a detectable label.

18. The antibody of claim 17, wherein the detectable label is selected from the group consisting of radioactive isotopes, enzymes, or chromophores.

19. A method of detecting a disease process associated with the degradation of fibrinogen in a mammal comprising testing a biological sample isolated from the mammal for the presence of a peptide having an unmasked fibrinogen epitope by contacting the blood sample isolated from the mammal with an antibody specific for the peptide and determining the presence of an antibody–peptide complex.

20. The method of claim 19, wherein the antibody recognizes an epitope comprising the amino acids 15 to 21 of the β–chain of human fibrinogen.

21. The method of claim 20, wherein the antibody is a monoclonal antibody.

FIG.1.

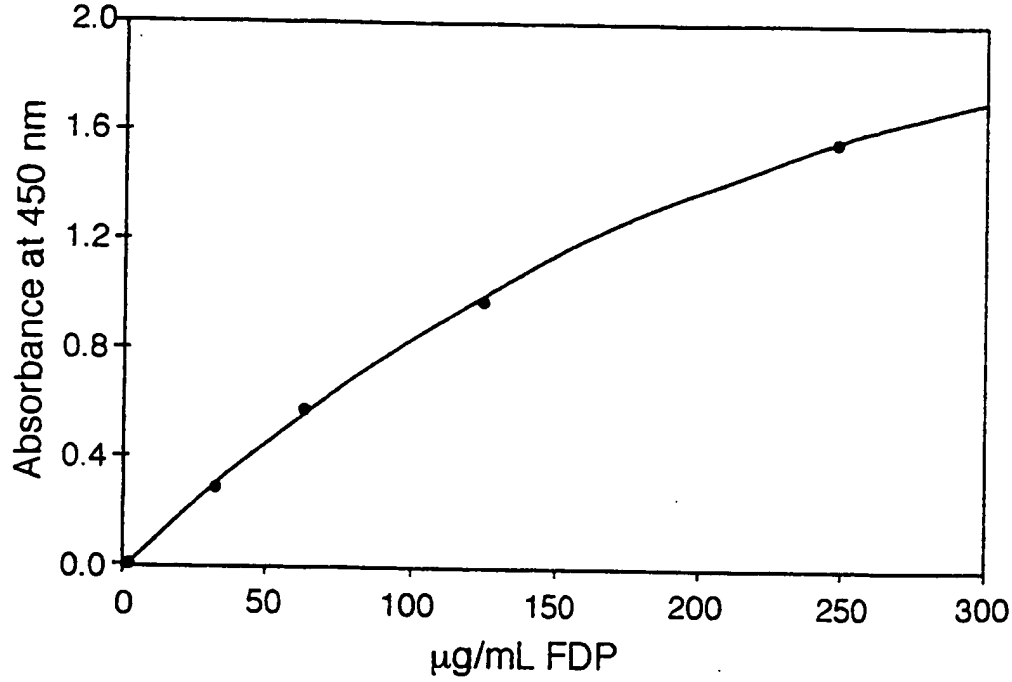
2.0
1.6
1.2
0.8
0.4
0.0
Absorbance at 450 nm
0
50
100
150
200
250
300
µg/mL FDP

FIG.2.

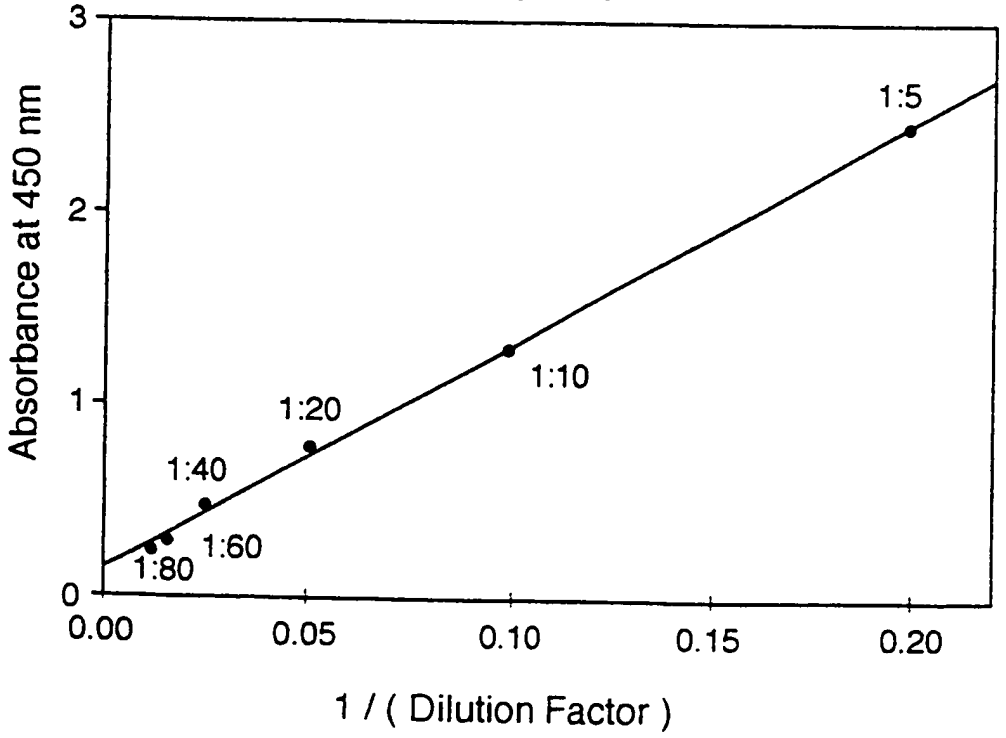
3
2
1
0
Absorbance at 450 nm
1:5
1:10
1:20
1:40
1:60
1:80
0.00
0.05
0.10
0.15
0.20
1 / (Dilution Factor)

FIG. 3.

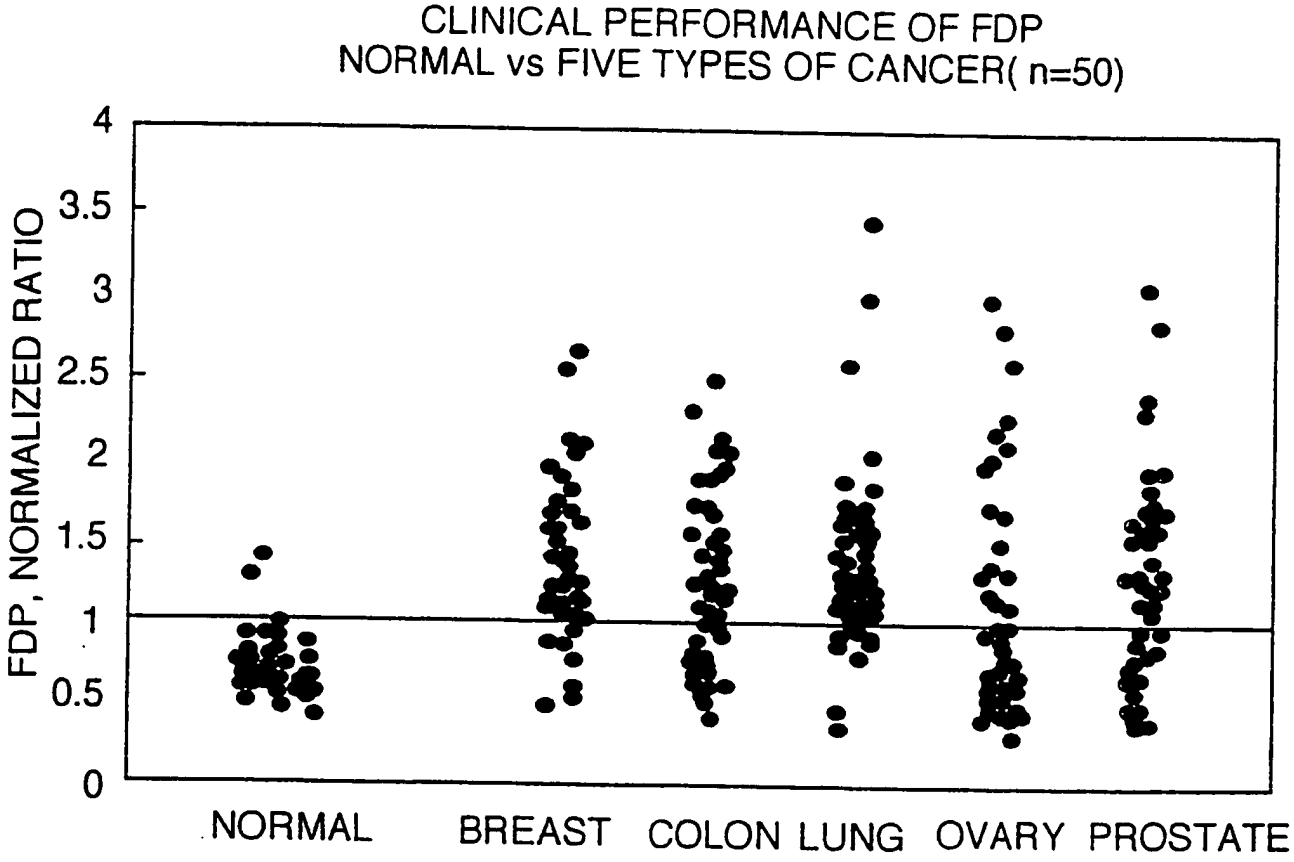
CLINICAL PERFORMANCE OF FDP
NORMAL vs FIVE TYPES OF CANCER(n=50)
FDP, NORMALIZED RATIO
4
3.5
3
2.5
2
1.5
1
0.5
0
NORMAL
BREAST
COLON
LUNG
OVARY
PROSTATE

FIG. 4.

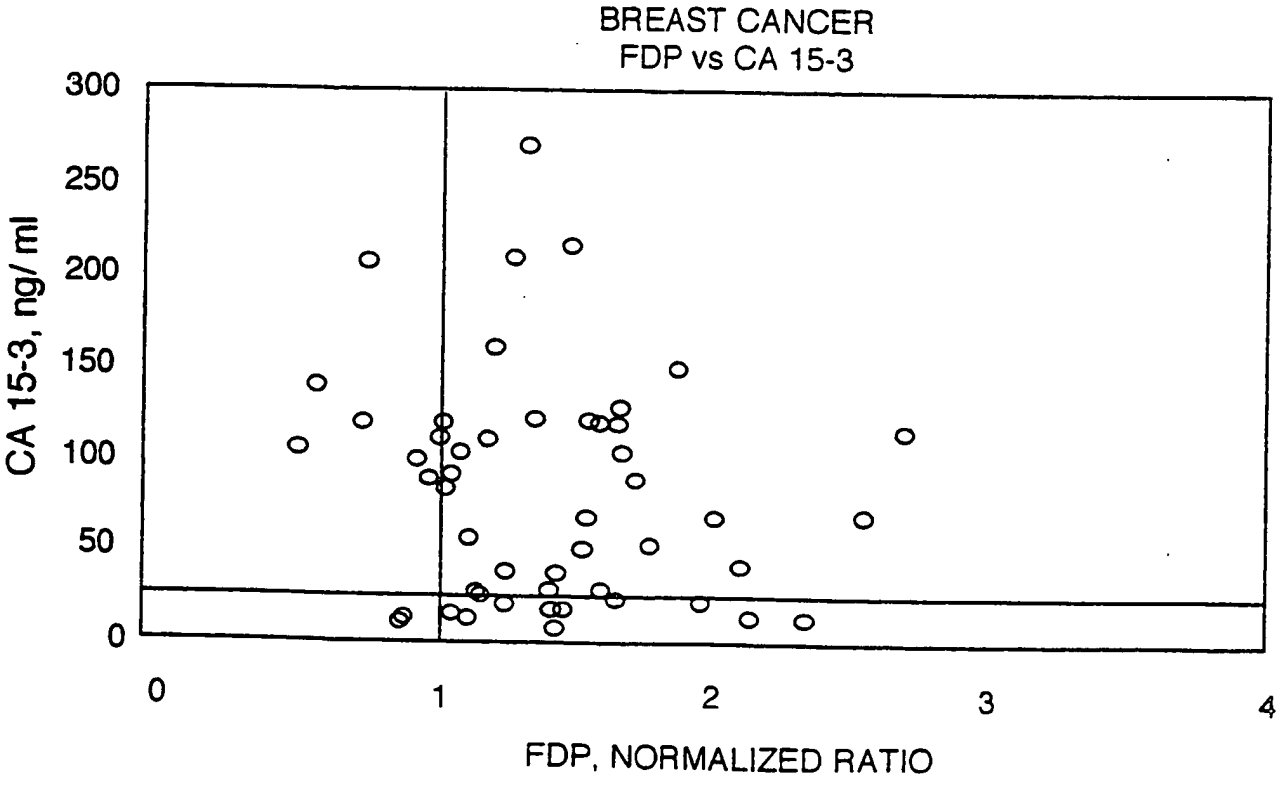
BREAST CANCER
FDP vs CA 15-3
CA 15-3, ng/ml
300
250
200
150
100
50
0
0
1
2
3
4
FDP, NORMALIZED RATIO

FIG. 5.

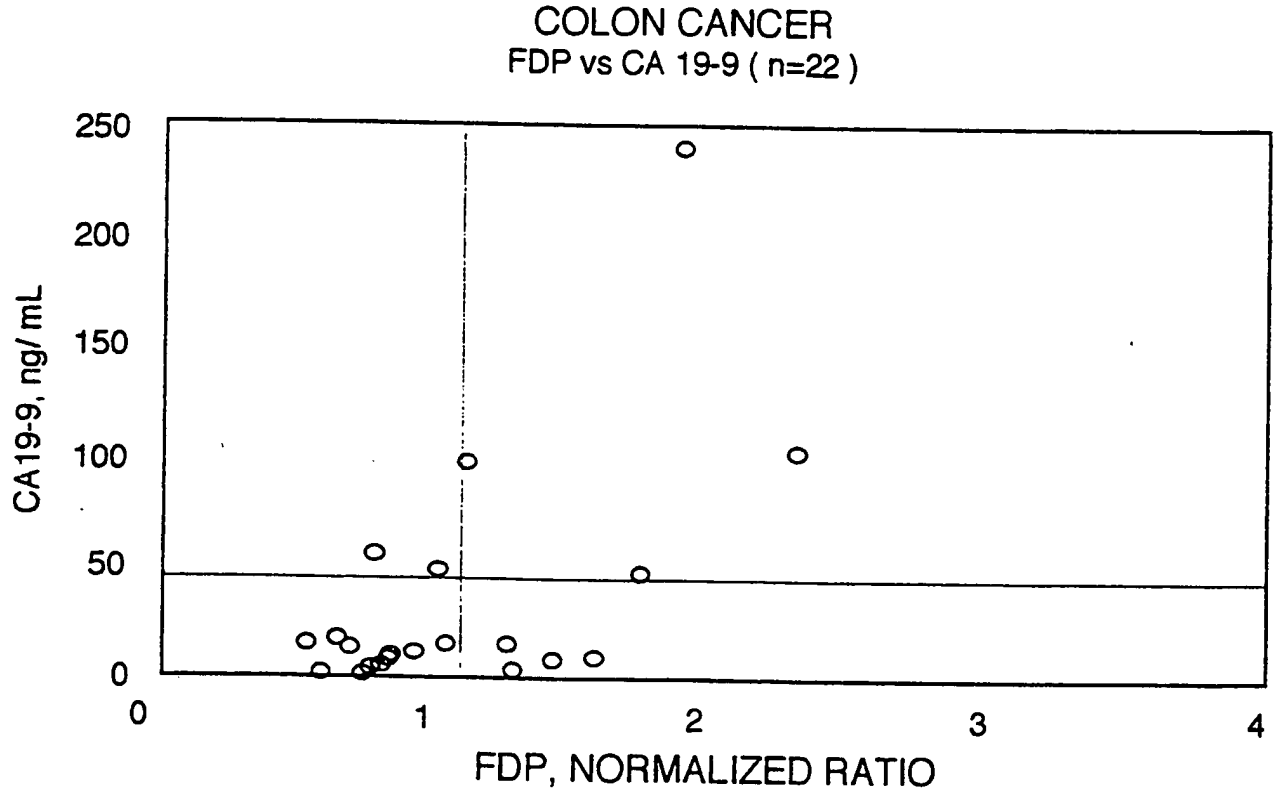
COLON CANCER
FDP vs CA 19-9 (n=22)
250
200
150
100
50
0
CA19-9, ng/mL
0
1
2
3
4
FDP, NORMALIZED RATIO

FIG. 6.

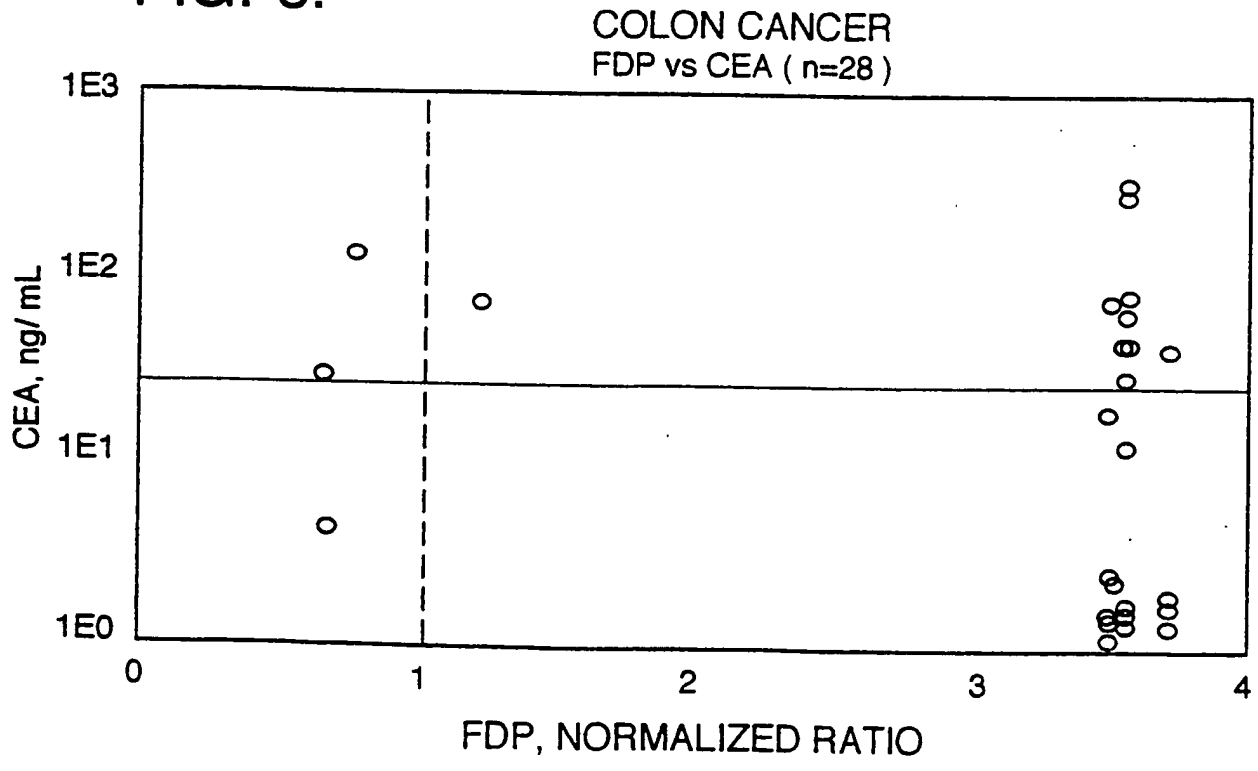
COLON CANCER
FDP vs CEA (n=28)
1E3
1E2
1E1
1E0
CEA, ng/mL
0
1
2
3
4
FDP, NORMALIZED RATIO

FIG. 7.

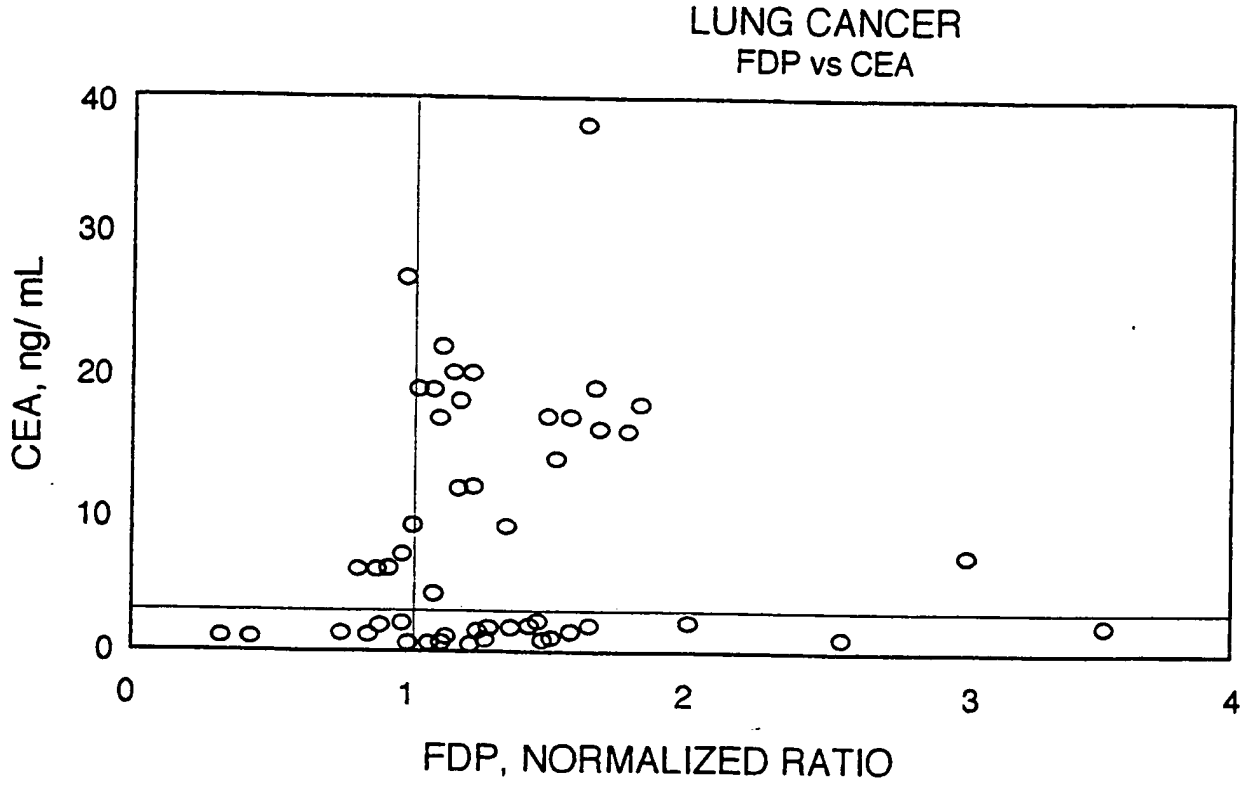
LUNG CANCER
FDP vs CEA
CEA, ng/ mL
40
30
20
10
0
0
1
2
3
4
FDP, NORMALIZED RATIO

FIG. 8.

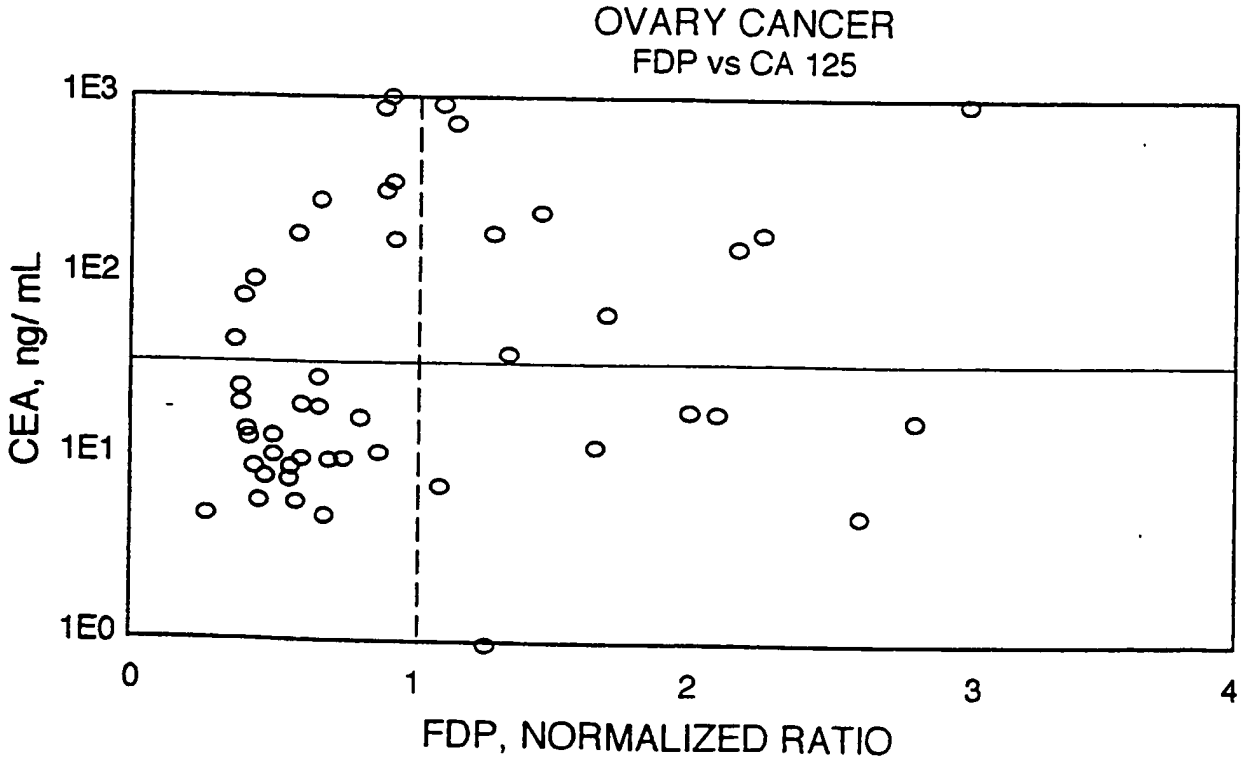
OVARY CANCER
FDP vs CA 125
CEA, ng/ mL
1E3
1E2
1E1
1E0
0
1
2
3
4
FDP, NORMALIZED RATIO

FIG. 9.

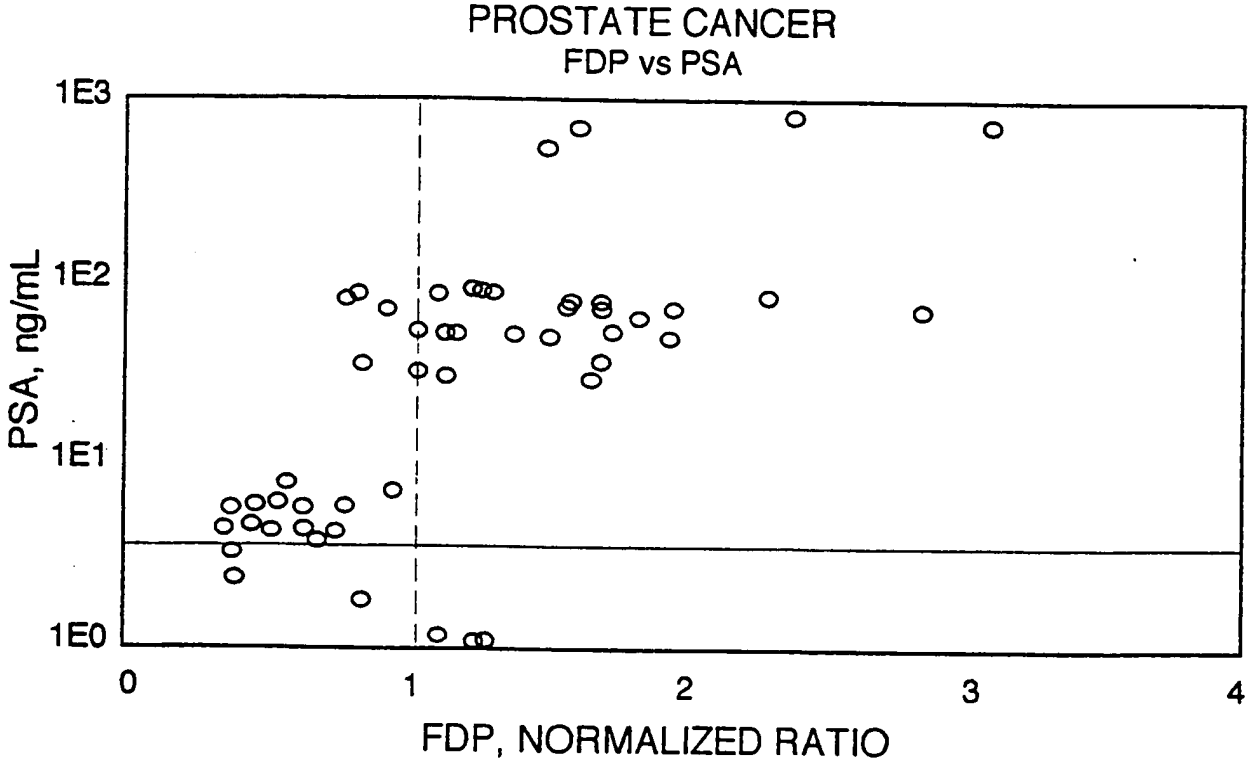
PROSTATE CANCER
FDP vs PSA
1E3
1E2
1E1
1E0
PSA, ng/mL
0
1
2
3
4
FDP, NORMALIZED RATIO

FIG. 10A

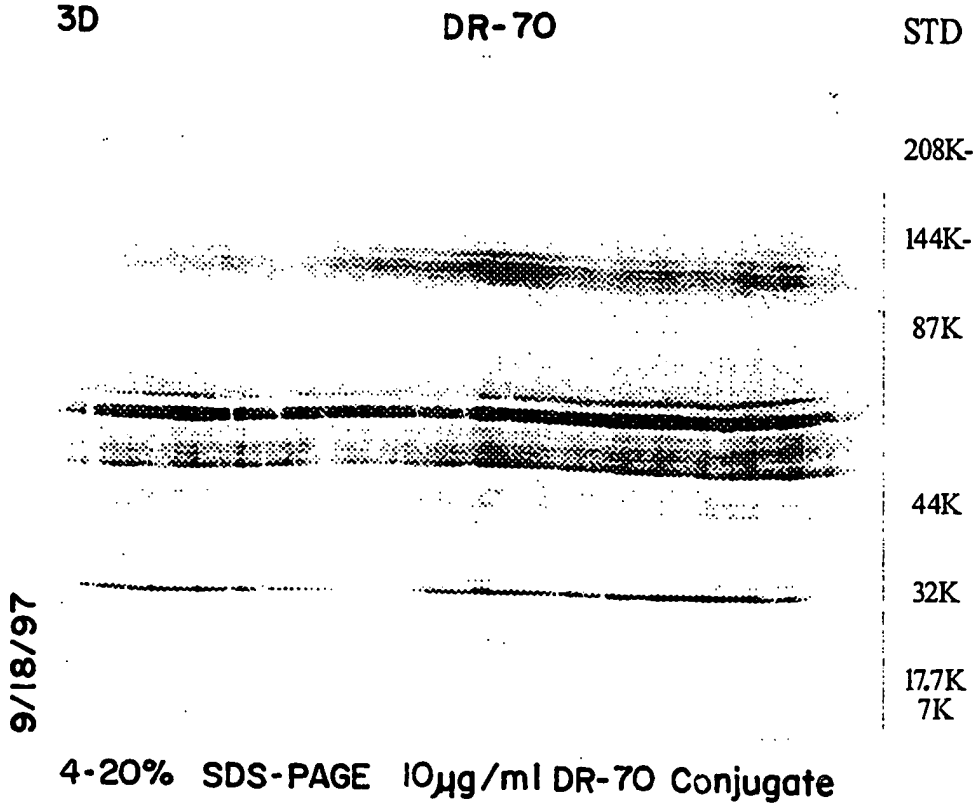

FIG. 10B

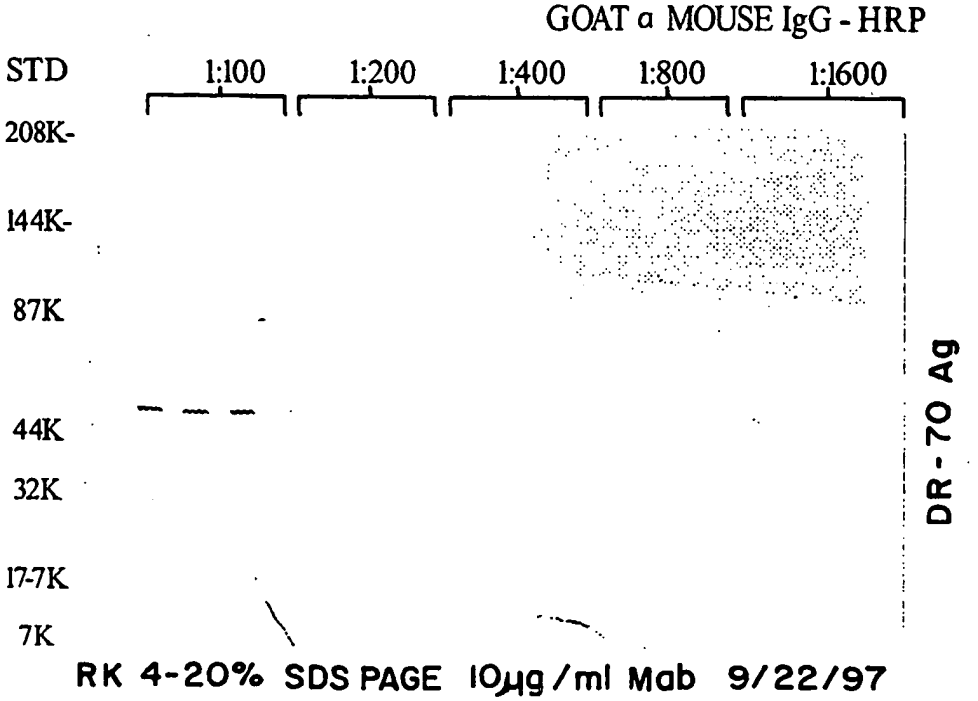

FIG. 10C

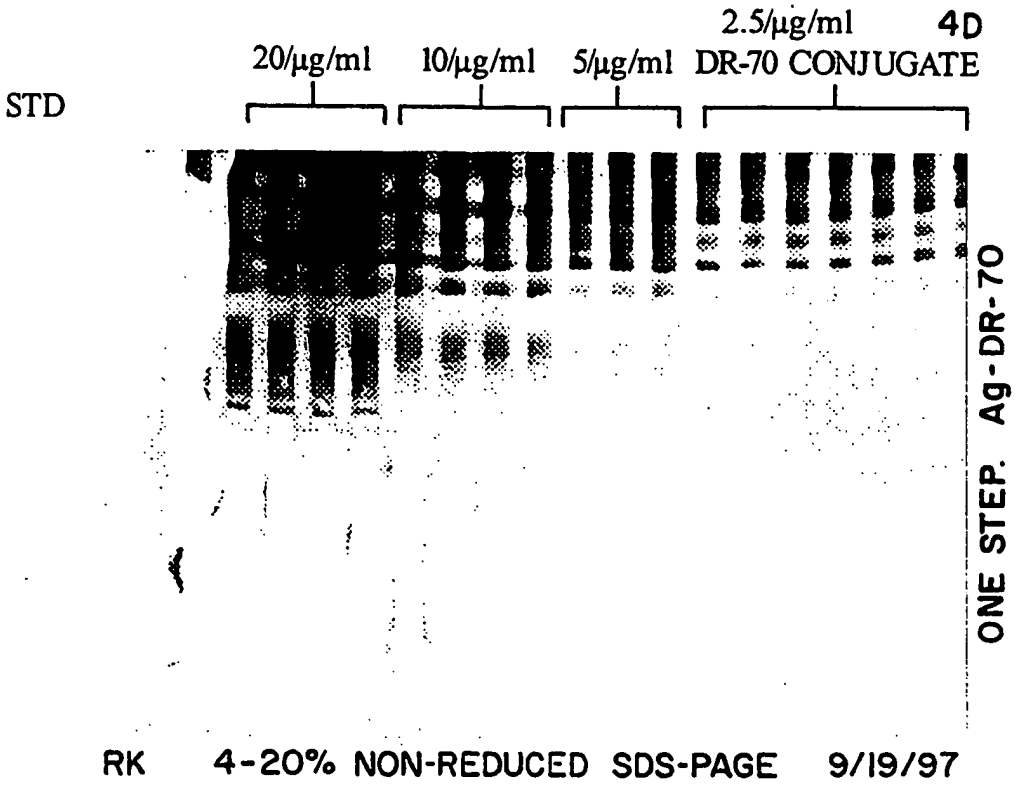

FIG. 10D

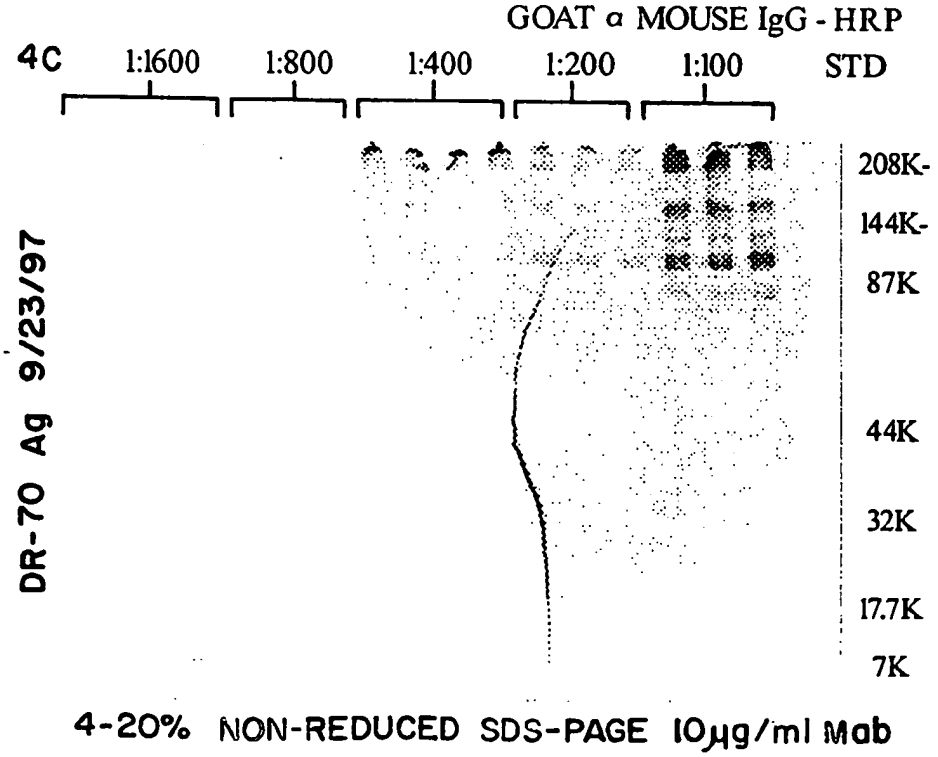

FIG. 11a.

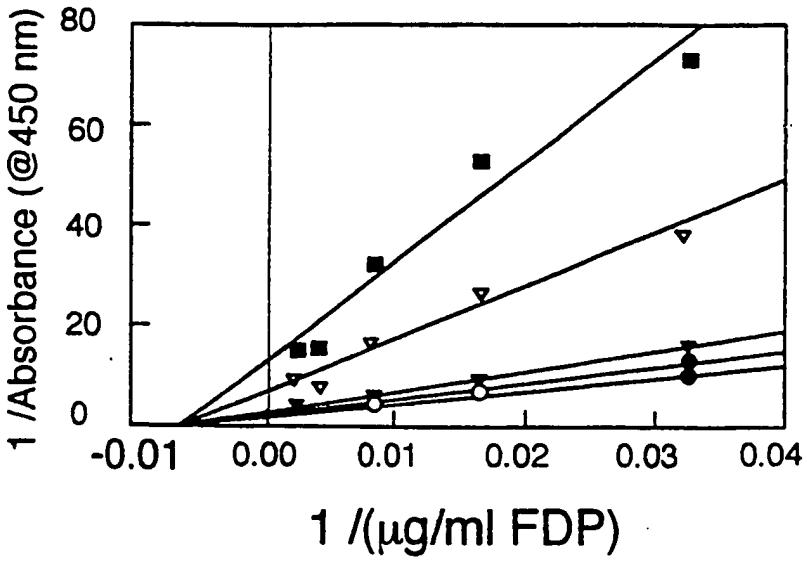

FIG. 11b.

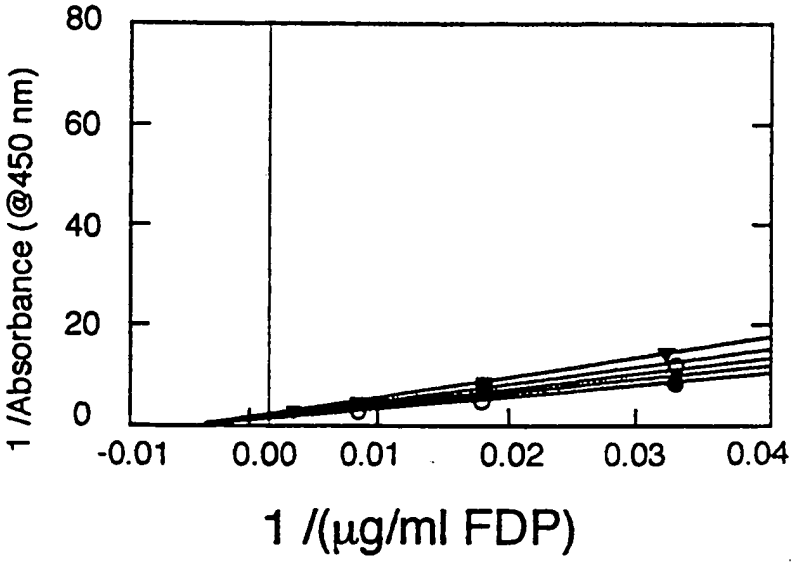

FIG. 11c.

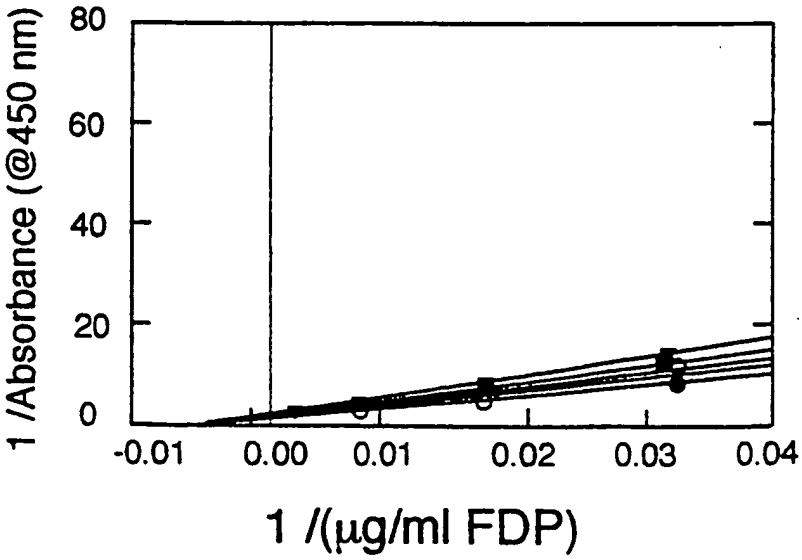

Figure 11. Selectivity of the Onochek assay: (A) noncompetitive inhibition by fibrinogen fragment D; (B) double reciprocal plot for fibrinogen; and (C) double reciprocal plot for fibrinogen fragment E.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5599678 A	04-02-1997	DE 4242736 A	23-06-1994
		AU 676859 B	27-03-1997
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		CA 2111645 A	18-06-1994
		EP 0605797 A	13-07-1994
		JP 6256388 A	13-09-1994

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 98/11162

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/574 C07K16/36

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J AMIRAL, M GROSLEY, F MIMILLA, V PLASSART, B CHAMBRETTE: "Monoclonal antibodies to different neo-epitopes on fibrinogen and fibrin degradation products" BLOOD COAGULATION AND FIBRINOLYSIS, vol. 1, no. 4-5, October 1990, pages 447-452, XP002080949	1,2, 5-11, 14-17,19
A	see the whole document	3,4,12, 13,20,21
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of theinternational search	Date of mailing of the international search report
19 October 1998	30/10/1998

Name and mailing address of the ISA	Authorized officer
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